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Creating interspecific hybrids with improved cold resistance in *Fragaria*

Gangjun Luo, Li Xue, Ruixue Guo, Yan Ding, Wenji Xu, Jiajun Lei*

College of Horticulture, Shenyang Agricultural University, Shenyang 110866, China

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ABSTRACT

The 12 interspecific hybridization combinations in *Fragaria* were performed to select promising individuals with improved cold resistance. The interspecific hybrids were obtained from the six combinations of Chandler ($8x$) × *F. viridis* ($2x$), Toyonoka ($8x$) × *F. viridis* ($2x$), Chandler ($8x$) × *F. orientalis* (♂) ($4x$), YH15-10 ($12x$) × *F. orientalis* (♂) ($4x$), AY175 ($10x$) × *F. moschata* (♂) ($6x$) and *F. moschata* (♀) ($6x$) × YH15-10 ($12x$), while failed in their reciprocal crosses. The chromosome number, morphological character and cold resistance of the interspecific hybrids and their parents were investigated. Five ploidy hybrids, including pentaploid ($2n = 5x = 35$), hexaploid ($2n = 6x = 42$), octaploid ($2n = 8x = 56$), enneaploid ($2n = 9x = 63$) and decaploid ($2n = 10x = 70$) were obtained. Only one ploidy hybrid was observed in each combination, except for the combination of YH15-10 × *F. orientalis* (♂), in which two ploidy hybrids ($8x$ and $10x$) were obtained. The identified chromosome number indicated that all of the observed individuals were true hybrids. Compared with the parents, most of the hybrids showed intermediate phenotype. The strong aromatic individuals were obtained, although most hybrids were sterile. The fertility of the hybrids from YH15-10 × *F. orientalis* (♂) was better than that of the other five combinations. Majority of the hybrids exhibited stronger cold resistance than lower cold resistant parent. For instance, 80.00% and 83.33% hybrids respectively from Toyonoka × *F. viridis* and Chandler × *F. orientalis* (♂) revealed superior cold resistance than the lower cold resistant parents ‘Toyonoka’ and ‘Chandler’. The five ploidy interspecific hybrids ($5x$, $6x$, $8x$, $9x$ and $10x$) with strong cold resistance obtained in this study were valuable resources for strawberry cultivar improvement.

1. Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is an herbaceous perennial plant that belongs to the genus *Fragaria* L., the family Rosaceae, which is one of the most important berry crops in the world (Deng and Lei, 2005; Staudt, 2009). For its rich nutrition, pleasant aroma and delectable flavor, strawberry is often used as eating and jam, and known as the ‘Berry Queen’. Furthermore, it also exhibits important values in juice and medicinal (Hummer and Hancock, 2009). In China, strawberry is one of the most important fresh fruits, and the fruit could harvest from November to next June. However, the strawberry cultivars exhibited a limited hardiness resistance in the cold climates (Rohloff et al., 2012). The winter damage resulted in 20% strawberry production losses per year in Northern China (Yang et al., 2010). Therefore, selecting new strawberry cultivar with improved cold resistance is a meaningful job for strawberry breeding programs.

Wide hybridization is an efficient way to create desirable horticultural traits (Lei et al., 2002). Cheng et al. (2010) obtained five interspecific hybrids ($2n = 4x = 36$) with excellent cold resistance from the cross of *Dendranthema morifolium* (Ramat.) Kitamura ‘rm20-12’ ($2n = 6x = 54$) × *D. nankingense* (Nakai) Tzvel. ($2n = 2x = 18$). Lian et al. (2016) obtained some interspecific hybrids ($2n = 2x = 32$) with attractive and larger flowers from the cross of *Iris dichotoma* ($2n = 2x = 32$) × *I. domestica* ($2n = 2x = 32$). Considering to strawberry, some breeders had created excellent characteristics via wide hybridization. Ahmadi and Bringhurst (1992) crossed *F. virginiana* ($8x$), *F. chiloensis* ($8x$) and *F. moschata* ($6x$) with *F. vesca* ($2x$), and doubled the pentaploid interspecific hybrids into decaploid individuals which were featured with strong disease resistance and pleasant flavor. Luo et al. (2017) selected some decaploid strawberries with strong cold tolerance and good flavor from the hybrids of Allstar ($8x$) × YH15-10 ($12x$).

The genus *Fragaria* has a basic chromosome number of seven ($x = 7$) and a diverse ploidy level ranging naturally from $2x$, $4x$, $5x$, $6x$, $8x$ to $10x$ (Hummer, 2012; Lei et al., 2017). The geographic distribution of *Fragaria* spans a wide range of Eurasia, North and South America, and East Asia (Johnson et al., 2014; Liston et al., 2014; Qiao et al., 2016). Wild strawberries revealed some value for breeding with strong

* Corresponding author.
E-mail addresses: gangjunluo@163.com (G. Luo), weixuelai@163.com (L. Xue), ruixueguo@163.com (R. Guo), dingyan0907@163.com (Y. Ding), iris_xuwenji@163.com (W. Xu), jiajunleisy@163.com (J. Lei).

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resistance and pleasant aroma. For example, hardiness resistance of *F. viridis*, *F. nubicola*, *F. mandschurica*, *F. orientalis* and *F. moschata*; waterlogging resistance of *F. nilgerrensis*, *F. viridis* and *F. pentaphylla*; aromatic fruit of *F. nilgerrensis*, *F. orientalis* and *F. moschata* (Bors and Sullivan, 2005a; Darrow, 1966; Hancock, 1990; Hummer et al., 2009; Staudt, 1989, 2009). Wild species have been utilized in breeding program to improve cultivated strawberry (Evans, 1977; Ge et al., 2016; Noguchi et al., 1997; Scott et al., 1972). Olbricht et al. (2006) obtained new plants with greater resistance to *Verticillium* from crossing *F.* × *ananassa* (8*x*) with *F. chiloensis* (8*x*). Noguchi et al. (2002) succeed in obtaining two fertile decaploid individuals by colchine treatment from two synthetic pentaploid individuals (Karen Berry (8*x*) × *F. nilgerrensis* (2*x*) and Toyonoka (8*x*) × *F. nilgerrensis* (2*x*)), and selected a peach-aromatic decaploid cultivar 'Tokun' with strong disease resistance from the hybrids of two decaploid individuals. Nowdays, the cultivar 'Tokun' is very popular in Japan and China. In this study, the cold resistant wild species *F. viridis* (2*x*), *F. orientalis* (4*x*) and *F. moschata* (6*x*) were used as materials crossed with strawberry cultivars ('Chandler' (8*x*) and 'Toyonoka' (8*x*)) and aromatic high-ploidy interspecific hybrids ('AY175' (10*x*) and 'YH15-10' (12*x*)) to select new strawberry germplasm with strong cold resistance.

2. Materials and methods

2.1. Plant materials

Wild strawberry species, *F. viridis* (2*x*) (Fig. 1a), *F. orientalis* (♀) (4*x*) (Fig. 1b), *F. orientalis* (♂) (4*x*) (Fig. 1c), *F. moschata* (♀) (6*x*) (Fig. 1d), *F. moschata* (♂) (6*x*) (Fig. 1e), strawberry cultivars 'Chandler' (8*x*) (Fig. 1f) and 'Toyonoka' (8*x*) (Fig. 1g), and aromatic high-ploidy interspecific hybrids 'AY175' (10*x*) (Fig. 1h) and 'YH15-10' (12*x*) (Fig. 1i) were used as hybridization parents. 'YH15-10' (12*x*) was derived from the cross of *F.* × *ananassa* cv. Yuhime (8*x*) × wild pentaploid strawberry accession 'Heilongjiang No. 7' (5*x*) with excellent cold resistance and aromatic flavor, and 'AY175' (10*x*) was derived from the cross of *F.* × *ananassa* cv. Allstar (8*x*) × YH15-10 (12*x*) with good cold resistance and aromatic fruit. All of the plants were grown in the open field of Strawberry Germplasm Resources Conservation Center, Shenyang Agricultural University, China.

2.2. Crossing and sowing

The 12 cross combinations were carried out in May 2015 as shown in Table 1. Each combination pollinated at least 30 flowers. Seeds were collected in June and stored in freezer at 4 °C. The seeds were sown in greenhouse in September and 10-week-old seedlings were transplanted to pots in December 2015, and planted in the open field on May 1, 2016.

2.3. Chromosome count

The chromosome of the 30 selected seedlings from each combination (all the hybrids were selected if the hybrid number of the combination was less than 30) and their parents was counted by the method of Lei et al. (2002) with few modifications. Young root tips were collected and pretreated with saturated p-dichlorobenzene for 3 h. The root tips were dissociated with 5 mol/L HCl for 10 min and washed with distilled water for 10 min, and then stained with carbol fuchsin for 30 min. The chromosome was observed and photographed under a BX51 Digital Microscope (Olympus, Japan).

2.4. Morphological characters investigation

The main traits of interspecific hybrids were observed, including fertility, stolon, plant height, flowers diameter, number of flowers per inflorescence, fruit shape, fruit color, fruit weight and soluble solids. The 30 interspecific hybrids were randomly selected from each combination. Each trait value represented the mean ± SE for three replicates.

2.5. Evaluation of cold tolerance

The evaluation method of cold tolerance was according to Houde et al. (2004) with slight modifications. The leaves were collected from the 30 seedlings in each combination in the open field in early September 2017. These leaves were placed on 1 cm thick of humid sand that prepared in petri dishes, and stored at 4 °C for 6 h. Then exposed to low temperatures of 0 °C, −4 °C, −8 °C, −12 °C, −16 °C, −20 °C, −24 °C, −28 °C and −32 °C for 10 h, respectively. The temperature was declined at a rate of 2 °C/h. Frozen leaves were thawed at 4 °C for 6 h. The electrical conductivity both room temperature (*R1*) and after boiling (*R2*) of each temperature treatment was measured by a conductivity meter (DDSJ-308A, China). Each value represented the mean of three replicates.

The relative conductivity (*REC*) = (*R1*/*R2*) × 100%. *REC* and temperature was fitted the logistic regression equation $y = K/(1 + ae^{-bx})$, where '*K*' represents the saturation capacity of cell damage rate, '*x*' is the treatment temperature, and *a* and *b* represents the equation parameters (Cheng et al. 2010). The LT_{50} was calculated by ln(1/a)/b. Each LT_{50} value represented the mean of three replicates.

2.6. Statistical analysis

The data were analyzed by one-way ANOVA using the software SPSS 17.0. The genetic analysis of the morphological characters, fruit quality and cold tolerance in the hybrids was investigated using both variation coefficient (*CV*) and broad sense heritability (H^2) analysis according to Xue et al. (2015).

3. Results

3.1. Fruit-setting rate and seed germination rate of the strawberry interspecific hybridization

The data obtained from 12 interspecific hybridization combinations were presented in Table 1. The cross combination Chandler (8*x*) × *F. viridis* (2*x*) indicated the highest fruit-setting rate (96.7%) and seed germination rate (74.2%) in the experiment, while no hybrid fruit was obtained from its reciprocal cross. The fruit-setting rates and seed germination rates of Toyonoka (8*x*) × *F. viridis* (2*x*) (70.0%, 71.5%), Chandler (8*x*) × *F. orientalis* (♂) (4*x*) (58.8%, 63.0%), YH15-10 (12*x*) × *F. orientalis* (♂) (4*x*) (52.0%, 8.0%) and AY175 (10*x*) × *F. moschata* (♂) (6*x*) (68.6%, 20.8%) were significantly higher than those of their reciprocal cross, respectively. But only five hybrids were obtained in *F. moschata* (♀) (6*x*) × YH15-10 (12*x*), and no hybrids were gained from its reciprocal cross.

3.2. Chromosome number of the strawberry interspecific hybrids

The somatic chromosome numbers of wild strawberry species *F. viridis* (Fig. 2a), *F. orientalis* (♂) (Fig. 2b), *F. moschata* (♀) and *F. moschata* (♂) (Fig. 2c) were 2*n* = 2*x* = 14, 2*n* = 4*x* = 28, 2*n* = 6*x* = 42 and 2*n* = 6*x* = 42, respectively, two cultivars 'Chandler' (Fig. 2d) and 'Toyonoka' were 2*n* = 8*x* = 56, and the two high-ploidy interspecific hybrids 'AY175' (Fig. 2e) and 'YH15-10' (Fig. 2f) were 2*n* = 10*x* = 70 and 2*n* = 12*x* = 84, respectively. All the 30 hybrids observed from the combinations of Chandler (8*x*) × *F. viridis* (2*x*) (hybrid 21-15, Fig. 2g) and Toyonoka (8*x*) × *F. viridis* (2*x*) (hybrid 22-16, Fig. 2h) were pentaploids (2*n* = 5*x* = 35). All the 30 hybrids observed from Chandler (8*x*) × *F. orientalis* (♂) (4*x*) were hexaploids (2*n* = 6*x* = 42) (hybrid 23-13, Fig. 2i). In the combination of AY175 (10*x*) × *F. moschata* (♂) (6*x*), all the 30 hybrids observed were octaploids (2*n* = 8*x* = 56)